No Negative Sublethal Effects of Two Insecticides on Prey Capture and Development of a Spider

Søren Toft* & Anders P. Jensen

Department of Zoology, University of Aarhus, Bldg. 135, DK-8000 Århus C, Denmark

(Received 23 January 1997; revised version received 4 August 1997; accepted 2 October 1997)

Abstract: Laboratory experiments were used to analyse the effect of two insecticides, dimethoate and cypermethrin, on prey capture, feeding and rate of development of the wolf spider *Pardosa amentata*. Though partly lethal doses (LD_{50} and LD_{10}) were applied and a high proportion of surviving spiders suffered paralysis for one to two days, which could be fatal under field conditions, no negative effects on other investigated biological characteristics (including development and predation rates) of the surviving individuals were observed. With very low doses of cypermethrin, killing rate and feeding of females in the period between the final moult and egg-sac deposition were increased compared to untreated controls. This is the first evidence of enhanced performance due to an insecticide in a spider. The possibility of insecticide stimulation of predator population recovery after eradication by spraying is discussed. © 1998 SCI.

Pestic. Sci., **52**, 223–228 (1998)

Key words: cypermethrin; dimethoate; development; feeding rate; functional response; hormesis; wolf spider; *Pardosa amentata*

1 INTRODUCTION

Spiders are known to be highly sensitive to insecticide spraying, populations often suffering mortalities of *c*.90%.[1–3] This sensitivity includes the dominant linyphiid species of agricultural fields, which are the ones most likely to experience a spraying event. These spiders seem particularly vulnerable to the synthetic pyrethroids.[3] Recovery of a spider population after spraying has been analysed primarily in terms of immigration from surrounding areas.[4,5] Little attention has been paid to the ecological performance of the part of the local population that survives the spraying. These animals may have received varying sublethal doses of the poison. How does this influence the predatory capacity of the individual spider, i.e. its potential as a biocontrol agent? How does it influence growth and development of these individuals and, subsequently, the recovery potential of the surviving population?

Most reported sublethal effects of insecticides are negative, i.e. they indicate reduced fitness of the treated individuals. However, the phenomenon of hormoligosis[6,7] or hormesis,[8] meaning an improved performance of individuals under chemical or other stresses, has been demonstrated in a wide variety of organisms. The general pattern is a dosage relationship, in which low doses of the poison have a stimulatory effect; at higher doses this changes to an inhibitory effect and finally becomes lethal. If this phenomenon occurs, great individual variation in responses to sublethal insecticide stress can be expected.

We wanted to study some of the possible sublethal effects of insecticides on spiders in the laboratory, focusing on aspects of behaviour and life history that are important for evaluating the importance of the spiders as biocontrol agents. Two series of experiments were performed. In one, we measured prey capture and rate

* To whom correspondence should be addressed.

Contract/grant sponsor: Danish Environmental Agency (Ministry of the Environment); Contract/grant number: 7041-0038.

of development of spiders as a function of insecticide (cypermethrin) dose. In the other, we analysed the functional response of individuals that survived partly lethal doses (LD_{50} or LD_{10}) of dimethoate or cypermetrin, compared to controls.

2 METHODS

Our test species was the wolf spider *Pardosa amentata* (Clerck), a common species in agricultural fields in northern Europe. The species has an annual life-cycle in Denmark with spring reproduction and hibernation mostly in the sub-adult instar (S. Toft, unpublished). For the experiments, spiders were collected from the field in the hibernating immature instar, either before or just after the winter. Experimental groups were created containing equal numbers of males and females and with approximately equal size (weight) variation; this served to reduce potential bias due to these factors. These groups were then randomly assigned to the different treatments. Wild-type fruit flies *Drosophila melanogaster* Meigen were used as prey in all experiments. Spiders were held in 120-ml plastic containers with a 2-cm bottom of plaster-of-paris with charcoal[9] wetted to maintain high humidity.

In all experiments insecticides or acetone were applied topically to the dorsal side of the abdomen of carbon dioxide-anaesthetized spiders using a Burkard microapplicator. To assure precision in application the treatment was performed under the dissection microscope. Pure, unformulated chemicals dissolved in acetone were used.

2.1 Experiment 1: Effect of different doses of cypermethrin on prey capture and development of the wolf spider *Pardosa amentata*

Preliminary experiments with animals of the same size as used here had established an LD_{50} of *c*.20 ng cypermethrin per spider and a 10% mortality for a dose of 10 ng. For the present experiment we chose 10, 1, 0·1 and 0·01 ng cypermethrin applied in a 1-μl drop of acetone as the series of experimental doses, with 1 μl of pure acetone applied to control animals. Each group contained at least ten individuals. As expected, one out of ten spiders in the highest-dose treatment died. With this dose there was also a strong knock-down effect: on the day following treatment six out of the nine survivors were immobile and might have been scored dead if not observed later; after two more days they had resumed seemingly normal activity. No knock-down effect was observed at other doses.

All 60 animals of the five groups were treated on the same day (day 0 of the experiment). Following this, or after recovery from knock-down, they were offered known numbers of fruit flies in excess of demand. The experiment was performed at 20°C and a 16 : 8 h light : dark regime, corresponding to spring conditions. The experimental containers were inspected every two days; the number of flies killed and/or eaten, as well as events like moulting and egg-laying were recorded, and the spiders were given fresh flies. The spiders were weighed before the start of the experiment and following the moult to adult and the exuvium was preserved in alcohol. At the end of the experiment all the spiders were preserved. As a measure of size, the length of the tibia of the first leg (tibia I) was measured and individual growth rates calculated (Dyar's ratio: length of tibia I of adult divided by length of tibia I of the subadult (measured on the exuvium)). Most of the spiders attained adulthood after one moult; three individuals that required two moults were deleted from the data analysis.

We had hoped to analyse also the effects of insecticides on the reproductive success of the females. Therefore, a male was kept with each female for two or three days, starting one or two days after her last moult. All females did produce an egg sac, but they were soon eaten by the females themselves, indicating that the eggs were unfertilized. Subsequent experiments (I. Rich & S. Mark, pers. comm.) showed that the females are unreceptive during the whole first week following the final moult.

2.2 Experiment 2: Effects of dimethoate and cypermethrin on the functional response of *Pardosa amentata*

The two insecticides were selected to represent different chemical groups of insecticides, the organophosphates and the synthetic pyrethroids. Both groups are neurotoxic, though acting through different physiological mechanisms,[10,11] thus potentially with different behavioural effects.[12]

For the dimethoate experiment a dose of 2·5 μg dimethoate dissolved in a 2·5-μl drop of acetone was applied to each experimental spider. According to preliminary experiments, this corresponded to LD_{50}. In the actual case 24 out of 49 spiders died (49%). Control spiders received a 2·5-μl drop of pure acetone; two out of 32 spiders died. There was a strong knock-down effect, from which survivors recovered within two days. None of the spiders was fed until the functional response measurements started two days after treatment.

In the cypermethrin experiment an LD_{10} was used, given as 10 ng cypermethrin in a 1-μl drop of acetone. Exactly six out of 60 treated animals died (10%); none of the control spiders, given a 1-μl drop of acetone, died. Otherwise the procedures of the two experimental series were identical.

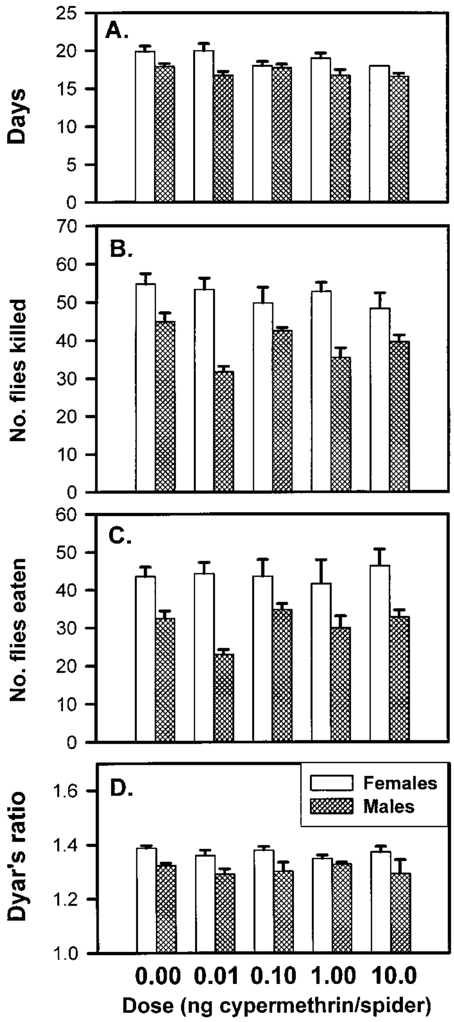

Fig. 1. Effects of different doses of cypermethrin applied topically on (A) the number of days from start of experiment to final moult, number of flies (B) killed or (C) eaten during the same period, and Dyar's ratio (tibia $I_{adult}/I_{sub\text{-}adult}$) (D) in the wolf spider *Pardosa amentata*. Average + 1 SE is shown (females $n = 29$, males $n = 27$).

Initial prey densities of 2, 3, 5, 7, 10 and 12 fruit flies per spider-container were established for both series; the dimethoate series additionally had a 15 flies per container density. Animals that moulted during the experiments were deleted from analysis, since feeding stops prior to a moult. Final total sample sizes were 40 (25 ctrl./15 exp.) for the dimethoate experiment and 42 (16 ctrl./26 exp.) for the cypermethrin experiment. For seven days the number of flies killed was noted every 24 h and live flies and prey remains were removed and replaced by new flies, so that densities were reset daily. Individual spiders remained at the same fly density treatment through all seven days. No adjustments have been made for treatment-unrelated fly mortality, since a separate test revealed this to be less than 1% in 24 h. The experiments were performed at 20°C and light : dark cycles of 12 : 12 h (autumn conditions) in the dimethoate experiment and 16 : 8 h (spring conditions) in the cypermethrin experiment.

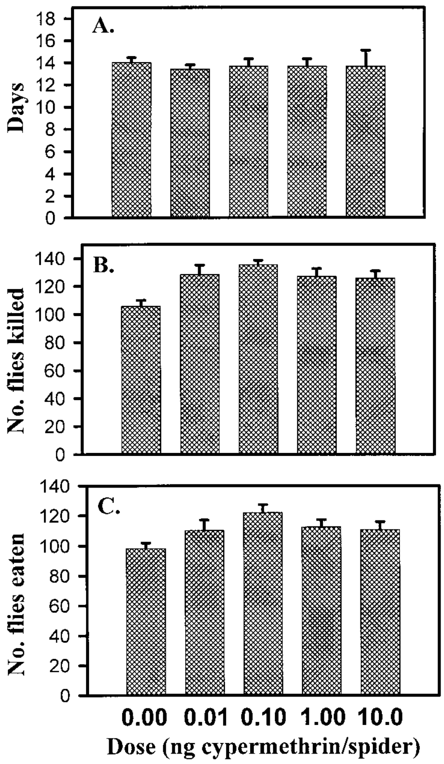

Fig. 2. Effects of different doses of cypermethrin applied topically on (A) the number of days from the final moult to laying of the first egg sac, and the number of flies (B) killed or (C) eaten during the same period in females of the wolf spider *Pardosa amentata*. Average + 1 SE is shown ($n = 26$).

Apart from comparing functional responses, the effects of insecticides on food uptake and utilization were analysed. Spiders were weighed at the start and at the end of the experiments. A dry weight: live weight ratio of 0·247 was then established by drying spiders in a vacuum oven at 60°C. In the same way an average dry weight of fruit flies and the weight of collected food remains were found. From this it was possible to calculate the dry weight of food obtained per fly killed (prey utilization) and the ratio of spider growth to food uptake (growth efficiency).

3 RESULTS

3.1 Experiment 1

Males needed fewer days than females to attain the adult stage (Fig. 1A, ANOVA $P = 0{\cdot}000$). It appears from Fig. 1A that cypermethrin-treated spiders developed slightly faster than the controls, though there was no statistically significant effect of cypermethrin ($P = 0{\cdot}333$). In agreement with this, the number of flies eaten during the period from treatment to adult moult shows no relationships to cypermethrin dose (Fig. 1C, $P = 0{\cdot}489$). The number of flies killed in the same period (Fig. 1B) shows a significant treatment effect for

males analysed separately ($P = 0{\cdot}004$) but not for females ($P = 0{\cdot}640$); however, the response is not strong enough to give a significant total effect ($P = 0{\cdot}059$). Treated males killed slightly fewer flies than control males (Fig. 1B).

Dyar's ratio was significantly larger for females than for males (Fig. 1D, $P = 0{\cdot}000$), with both initial body weight and sub-adult tibia I as positive, significant covariates ($P = 0{\cdot}009$ and $0{\cdot}000$, respectively). It was independent of cypermethrin treatment ($P = 0{\cdot}240$).

The egg development time, i.e. the time from the last moult to laying of the first egg sac, was also uninfluenced by cypermethrin treatment (Fig. 2A, $P = 0{\cdot}818$). In spite of the fact that the spiders were selected to be as homogenous as possible with respect to size, the body weight at the adult moult was a significant covariate ($P = 0{\cdot}041$). The total number of flies killed during the egg development period showed a significant treatment effect (Fig. 2B, $P = 0{\cdot}018$). Treated spiders killed more flies than control spiders. A multiple regression revealed no relationship to dosage, however. As can be seen from Fig. 2B, this is because the treatment effect is due to high killing rates mainly at the low (0·01 and 0·1 ng) cypermethrin doses. The graph of numbers of flies eaten during the egg developmental period (Fig. 2C) is very similar to Fig. 2B, but the effect is statistically less well supported. However, if all cypermethrin-treated spiders are tested *en bloc* against control spiders, we find a significant effect of the insecticide ($P = 0{\cdot}019$) with body weight at moult as a significant covariate ($P = 0{\cdot}027$). Treated spiders ate more flies than control spiders.

3.2 Experiment 2

No significant effects of insecticide treatment on prey capture was found in either experimental series (Tables 1, 2). Obviously, prey density is an important determinant of prey capture, though statistical significance seems to disappear over time. This is due to the spiders at the high densities becoming satiated during the experiment, while those at the low densities remain hungry. Early in the experiment the functional response curves were clearly of Holling type 2,[13] i.e. increasing towards an asymptote. Later they became Holling type 4, i.e. bell-shaped, and, at the end, relationships disappeared completely.

Neither the amount of food extracted per fly killed, nor the growth efficiency of the spiders was influenced by insecticide treatment in any of the two experimental series (Tables 1, 2). In the dimethoate experiment, prey

TABLE 1

Effects of Topical Dimethoate Treatment (LD_{50}) and Prey Density (2, 3, 5, 7, 10, 12 and 15 Fruit Flies per Spider Container) on the Number of Flies Killed per 24 h, Prey Utilization (mg Food Extracted per Fly) and Growth Efficiency (mg Weight Gain per mg Food Eaten) by the Wolf Spider *Pardosa amentata* over Seven Consecutive Days

	No. flies killed									
Day	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Total*	*Prey utilization*	*Growth efficiency*
Dimethoate	0·273	0·060	0·900	0·541	0·774	0·246	0·235	0·827	0·214	0·939
Prey density	0·000[a]	0·000[a]	0·093	0·032[b]	0·126	0·470	0·230	0·001[a]	0·035[c]	0·861

[a] No. of flies killed increases with fly density (basically a type 2 functional response), though with a peak at 12 flies per container.
[b] No. of flies killed peaks at seven flies per container (type 4 functional response).
[c] Prey utilization decreases with prey density.
Numbers show P values for the two factors in two-way ANOVAs. There were no significant treatment*prey density interactions.

TABLE 2

Effects of Topical Cypermethrin Treatment (LD_{10}) and Prey Density (2, 3, 5, 7, 10 and 12 Fruit Flies per Spider Container) on the Number of Flies Killed per 24 h, Prey Utilization (mg Food Extracted per Fly) and Growth Efficiency (mg Weight Gain per mg Food Eaten) by the Wolf Spider *Pardosa amentata* over a Seven-Day Experimental Period

	No. flies killed									
Day	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Total*	*Prey utilization*	*Growth efficiency*
Cypermethrin	0·345	0·791	0·405	0·901	0·770	0·546	0·649	0·495	0·541	0·647
Prey density	0·000[a]	0·069	0·092	0·021[b]	0·457	0·321	0·318	0·014[a]	0·592	0·002[c]

a No. of flies killed increases with fly density (type 2 functional response).
[b] No. of flies killed peaks at five flies per container (type 4 functional response).
[c] Growth efficiency decreases with prey density.
Numbers show P-values for the two factors in two-way ANOVAs. There were no significant treatment*prey density interactions.

density significantly influenced prey utilization. At high availability the spiders extracted less food per prey item. In the cypermethrin experiment, the growth efficiency of the spiders decreased with increasing prey density. Also, females captured significantly more flies than males on five out of the seven days. Prey utilization did not differ between the sexes; however, change in dry weight over the experimental period and growth efficiency were both higher for females than for males ($P = 0{\cdot}005$ and 0·036, respectively).

4 DISCUSSION

In spite of the fact that very high doses were used in all experiments, the effects of the two insecticides on the performance of the spiders were surprisingly small. The highest dose in Exp. 1 and the doses used in Exp. 2 were chosen to be lethal to some of the treated spiders and they had strong knock-down effects on those that eventually survived. We had a clear expectation of negative effects on all the measured parameters. However, in Exp. 1 development was faster (though not significantly) rather than slowed down, even though the spiders at the highest dose spent one or two days in coma. Also in Exp. 2 there was no indication of reduced prey capture rate at any prey density, though the experimental spiders had just recovered from the knock-down due to the high doses used. In general, most of our comparisons show no significant treatment effects. The few significant effects we got were contrary to expectations. Since there are several statistically significant effects with respect to other factors (sex, prey density etc.), the lack of significance for insecticide treatment cannot be explained simply by insufficient sample sizes. We must therefore conclude that these spiders suffered no, or at least only minor, ill-effects with respect to the parameters measured here, once they had recovered from insecticide treatment, even though treated with close to lethal doses.

We present statistically significant, though numerically small stimulatory effects of low doses of cypermethrin on killing and feeding rate of mature females. These effects are similar to the phenomenon of hormesis, which is known from a wide range of organisms, including insects,[7,8] but has not been demonstrated in spiders before. We are also unaware of similar findings for other predatory anthropods. Increased fecundity has repeatedly been demonstrated as a hormetic effect in spider mites.[14,15] In accordance with the general theory of hormesis, the effects were found only at very low doses of the insecticide.[6,8] Dutt[16] found increased rate of development in a beetle even with insecticide concentrations of LC_{20}–LC_{40}. In the field, spiders hit by high sublethal doses, causing knock-down, are likely to die of desiccation or predation.[17] Thus, the effects of low sublethal doses are the most important ones in practice.

Improved fecundity as a result of hormesis has been proposed as a possible mechanism explaining the often rapid resurgence of a pest population following spraying.[18,19] The simultaneous eradication of the natural predators of the pest would allow the full realization of this potential for increase. If hormetic stimulation turns out to be widespread also among natural enemies, it would tend to reduce this advantage of the pest. In the first place this might be due to increased attack rates of the predators (demonstrated here), in the longer run to a possibly increased reproductive rate as a result of an expectedly increased fecundity (not shown here). However, the low magnitude of the effects probably does not allow the predators to keep up with the pests. Much more detailed information is needed before this question can be properly evaluated.

ACKNOWLEDGEMENTS

The study was supported by grant no. 7041-0038 from the Danish Environmental Agency (Ministry of the Environment).

REFERENCES

1. Vickerman, G. P. & Sunderland, K. D., Some effects of dimethoate on arthropods in winter wheat. *J. Appl. Ecol.*, **14** (1977) 767–77.
2. Basedow, T., Rzehak, H. & Voß, K., Studies on the effect of deltamethrin sprays on the numbers of epigeal predatory arthropods occurring in arable fields. *Pestic. Sci.*, **16** (1985) 325–31.
3. Stark, J. D., Jepson, P. C. & Thomas, C. F. G., The effects of pesticides on spiders from the lab to the landscape. *Rev. Pestic. Toxicol.*, **3** (1995) 83–110.
4. Jepson, P. C. & Thacker, J. R. M., Analysis of the spatial component of pesticide side-effects on non-target invertebrate populations and its relevance to hazard analysis. *Funct. Ecol.*, **4** (1990) 349–55.
5. Duffield, S. J. & Moffatt, J., Does scale influence the recovery of dimethoate-depleted invertebrate populations in winter wheat? In *Behaviour and Impact of Aphidophaga*, ed. L. Polgár, R. J. Chambers & A. F. G. Dixon. SPB Academic Publishing BV, The Hague, The Netherlands, 1991, pp. 305–11.
6. Luckey, T. D., Antibiotic action in adaptation. *Nature (London)* **198** (1963) 263–5.
7. Luckey, T. D., Insecticide hormoligosis, *J. Econ. Entomol.*, **61** (1968) 7–12.
8. Stebbing, A. R. D., Hormesis—the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.*, **22** (1982) 213–34.
9. Usher, M. B. & Stoneman, C. F., *Folsomia candida*—an ideal organism for population studies in the laboratory. *J. Biol. Educ.*, **11** (1977) 83–90.
10. WHO, *Cypermethrin*, *Enviromental Health Criteria*, **82**, Geneve, 1989.
11. WHO, *Dimethoate*, *Environmental Health Criteria*, **90**, Geneve, 1989.
12. Haynes, K. F., Sublethal effects of neurotoxic insecticides on insect behaviour. *Ann. Rev. Entomol.*, **33** (1988) 149–68.

13. Crawley, M. J., *Natural Enemies*. Blackwell Scientific Press, Oxford, 1992.
14. Dittrich, V., Streibert, P. & Bathe, P. A., An old case reopened: mite stimulation by insecticide residues. *Environ. Entomol.*, **3** (1974) 534–40.
15. Jones, V. P. & Parrella, M. P., The sublethal effects of selected insecticides on life table parameters of *Panonychus citri* (Acari: Tetranychidae). *Can. Ent.*, **116** (1984) 1033–40.
16. Dutt, N., Bioecological studies on F_1 immature forms obtained from crosses of *Callosobruchus chinensis* L. exposed to sublethal concentrations of organophosphorus insecticides. *Indian J. Ent.*, **50** (1988) 307–14.
17. Everts, J. W., The toxic effects of deltamethrin on linyphiid and erigonid spiders in connection with ambient temperature, humidity and predation. *Arch. Environ. Contam. Toxicol.*, **20** (1991) 20–24.
18. Bartlett, B. R., Outbreaks of two-spotted spider mites and cotton aphids following pesticide treatment. I. Pest stimulation versus natural enemy destruction. *J. Econ. Entomol.*, **61** (1968) 297–303.
19. Huffaker, C. B., Vrie, M. van de & McMurtry, J. A., Ecology of tetranychid mites and their natural enemies. II. Tetranychid populations and their possible control by predators: an evaluation. *Hilgardia*, **40** (1970) 391–458.